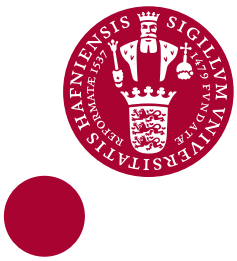

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Early development of the porcine embryo: the importance of cell signalling in development of pluripotent cell lines

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Abstract. Understanding the cell signalling events that govern cell renewal in porcine pluripotent cells may help improve culture conditions and allow for establishment of bona fide porcine embryonic stem cells (pESC) and stable porcine induced pluripotent stem cells (piPSC). This review investigates cell signalling in the porcine preimplantation embryo containing either the inner cell mass or epiblast, with particular emphasis on fibroblast growth factor, SMAD, WNT and Janus tyrosine kinases/signal transducers and activators of transcription signalling. It is clear that key differences exist in the cell signalling events that govern pluripotency in this species compared with similar embryonic stages in mouse and human. The fact that bona fide pESC have still not been produced and that piPSC cannot survive in culture following the silencing or downregulation of the reprogramming transgenes suggest that culture conditions are not optimal. Unravelling the factor/s that regulate pluripotency in porcine embryos will pave the way for future establishment of stable pluripotent stem cell lines.

Additional keywords: cell signalling, embryo, FGF pathway, JAK/STAT pathway, pluripotent stem cells, porcine, preimplantation, SMAD pathway, WNT pathway.

Introduction

The pig is currently an under-represented model for biomedical studies, but is steadily gaining acceptance as a potential alternative model for studying disease and testing of pharmaceutical products, as well as in regenerative medicine. This is primarily due to the development of new pig models of disease (Rogers *et al.* 2008; Kragh *et al.* 2009; Renner *et al.* 2010; Klymiuk *et al.* 2012; Luo *et al.* 2012; Staunstrup *et al.* 2012), good characterisation of physiological conditions (in particular within miniature pig breeds; Jacobs 2006; Bode *et al.* 2010; McAnulty *et al.* 2011) and increased research into embryo technologies and the cell culture of porcine cells (Oestrup *et al.* 2009; Esteban *et al.* 2010; Gil *et al.* 2010; Zhao *et al.* 2010).

In the case of regenerative medicine, the pig has become of particular interest given the recent breakthroughs in developing *in vitro* cell models, including porcine (p) induced pluripotent stem cells (iPSCs; Roberts *et al.* 2009; Esteban *et al.* 2010), skin-derived progenitor cells (Zhao *et al.* 2009; Lermen *et al.* 2010), neural stem/progenitor cells (Uebing-Czipura *et al.* 2008; Liard *et al.* 2009; Puy *et al.* 2010; Rasmussen *et al.* 2011; Yin *et al.* 2011; Zhao *et al.* 2012), peripheral blood-derived multipotent adult progenitor cells (Price *et al.* 2006; Spitzer *et al.* 2011), primordial germ cells (Petkov *et al.* 2011) and mesenchymal stem cells (Rho *et al.* 2009; Monaco *et al.* 2011; Miernik and Karasinski 2012). These cells have the capacity to be cultured *in vitro* and be differentiated into multiple, specific cell types that can be used for studying disease and could be considered for future cell therapy. Furthermore, with the recent development of severe combined immunodeficient pigs lacking interleukin (IL)-2 receptor γ (IL2rg), this animal model already presents an excellent step towards developing a model for xenotransplantation studies and for studying diseases, such as human cancers (Suzuki *et al.* 2012).

Despite these breakthroughs, certain porcine cells have been difficult to culture compared with similar cells obtained from mouse and human. For example, culture of porcine (p) embryonic stem cells (ESCs) has been a particularly difficult task (Brevini *et al.* 2007*a*; Hall 2008; Muñoz *et al.* 2009). The ESCs are derived from either the inner cell mass (ICM) or epiblast (EPI) of the developing preimplantation blastocyst and, to date, no bona fide cell lines from the pig have been reported that recapitulate the features of mouse (m) ESCs (i.e. can be cultured indefinitely *in vitro*, form cells representative of all three germ layers *in vitro* and *in vivo* and can contribute to the germline). Induced pluripotent stem cell lines are reprogrammed somatic cells that recapitulate pluripotent ESC and were first produced in the pig in 2009 (Esteban *et al.* 2009). Since then, only one research group has reported that these cells can generate chimeras (West *et al.* 2010) and contribute to the germline (West *et al.* 2011); however, chimera production was found to be low and germline transmission even lower. Despite the recent advances in the generation of piPSC, these cells are unable to silence the inserted transgenes and, if forced, the repression of these transgenes results in the loss of the ability of

the piPSC to self-renew in culture (Esteban *et al.* 2009; Ezashi *et al.* 2009; Roberts *et al.* 2009; West *et al.* 2010; Montserrat *et al.* 2011; Hall *et al.* 2012). Reasons for these difficulties have been addressed by many and may be accounted for by differences in embryo development between species (Hall 2008). It is anticipated that piPSC may help simplify the production of transgenic pigs, with the aid of and in combination with more modern transgenic approaches and genetic engineering (Fahrenkrug *et al.* 2010; Garrels *et al.* 2012), such as the use of transposons and recombinases (Clark *et al.* 2007). In addition, despite the recent improvements in the culture of porcine embryos and media composition (Dang-Nguyen *et al.* 2011; Nguyen *et al.* 2011; Yoshioka 2011; Liu *et al.* 2012; Tareq *et al.* 2012), retaining the pluripotency of either porcine ICM or EPI cells *in vitro* has been very difficult.

Pluripotent stem cell proliferation and cell renewal is driven by complex cell signalling cascades. These cascades differ markedly between mouse and human pluripotent cells, as does the composition of the media needed to maintain cell pluripotency *in vitro*. Furthermore, ESCs are in a dynamic equilibrium, which can be observed by the heterogeneous expression of genes such as *Nanog* and *Stella* (Chambers *et al.* 2007; Hayashi *et al.* 2008). These cascades also differ depending on the embryonic stage the stem cells are recovered from. Recently, proteomic profiling of mESC, epiblast stem cells (epiSC), trophoblast stem cells and extraembryonic endoderm stem cells revealed that unique cell signalling exists on the cell plasma membrane between these different stem cell types (Rugg-Gunn *et al.* 2012). The culture medium is paramount for maintaining a particular stem cell state and may even radically alter cell fate under the right conditions. This has been shown recently following the reprogramming of mouse fibroblasts into a pluripotent stem cell state for a short transitional period and their subsequent conversion into neural cells simply by growing the reprogrammed cells in a neural-based medium (Kim *et al.* 2011). Given that bona fide pESC have not been established from any embryonic stage, this infers that culture conditions may not be optimal. This article provides an overview of the cell signalling events known in the developing porcine blastocyst, with special emphasis on the porcine ICM, EPI and trophectoderm (TE). It is anticipated that further studies that can identify cell signalling regulating porcine pluripotency may help improve the culture conditions needed to sustain the growth and retain the pluripotent state of derived pluripotent stem cells *in vitro*.

Regulation of pluripotency in mouse and human pluripotent stem cells

More is known about cell signalling governing pluripotency within mESC and human (h) ESC. It has been determined that two modules exist to regulate pluripotency and cell renewal: the Octamer-binding transcription factor 4 (Oct4)-centric and Myc-centric modules. These two modules are multiprotein complexes that are activated downstream of important cell signalling pathways and include several different transcription factors in each. In particular, Oct4, sex-determining region Y-box2 (Sox2), Nanog, mothers against DPP homologue 1 (Smad1), signal transducer and activator of transcription 3 (Stat3) and Tcf3 (Chen *et al.* 2008; Cole *et al.* 2008) are known to form the Oct4-centric module, whereas c-Myc, n-Myc, E2f1, Zfx, Rex1 and Ronin are found in the Myc centric module (Chen *et al.* 2008; Kim *et al.* 2008; Dejosez *et al.* 2010). Despite the fact that both species have Oct4, Nanog and Sox2 as major transcriptional factors that regulate self-renewal (Niwa *et al.* 2000; Chambers *et al.* 2007; Masui *et al.* 2007), recent studies have shown that Oct4 and Nanog bind to different elements in these species. In hESC, OCT4 and NANOG bind to endogenous retroviral sequence 1-repeat transposable elements (ERV1), whereas in mESC these factors bind to murine-specific endogenous retrovirus K-repeat elements (ERVK; Bourque *et al.* 2008; Kunarso *et al.* 2010). A recent report confirms that OCT4, NANOG and SOX2 in hESC control specific cell fate, with OCT4 being shown to modulate four different developmental cell fates and NANOG and SOX2 repressing ectoderm and mesendoderm differentiation, respectively (Wang *et al.* 2012). Investigations of the post-translational activity of these transcription factors in the mouse has shown that regulation of pluripotency is accomplished by Oct4 binding DNA in multiple heterodimer and homodimer configurations by rapid alteration of activation in response to varying extracellular signals (Saxe *et al.* 2009). Nanog has been shown to dimerise through its C-terminal domain rather than its homeodomain (Wang *et al.* 2008).

There are considerable differences in cell signalling between mESC and hESC. For example, mESC pluripotency is primarily regulated by Janus tyrosine kinases (JAK)/signal transducers and activators of transcription (STAT) signalling, but WNT and bone morphogenetic protein (BMP) signalling is also important in maintaining stemness (Ng and Surani 2011). The transcriptional regulation of pluripotency of hESC differs and, moreover, depends on fibroblast growth factor (FGF) and transforming growth factor (TGF)-β/ACTIVIN/NODAL signalling (Ng and Surani 2011). It is well known that supplementation of the medium can sustain pluripotent stem cells *in vitro*; for example, basic (b) FGF/FGF-2 in the case of hESC and leukaemia inhibitory factor (LIF) in the case of mouse ESC, which activate the mitogen-activated protein kinase (MAPK) pathway in hESC (Eiselleova *et al.* 2009) and the JAK/STAT, phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) and Src homology 2 domain-containing tyrosine phosphatase 2 (SHP2)/MAPK pathways in mESC (Hirai *et al.* 2011). In addition, bFGF has been shown to activate extracellular signal-regulated kinase (ERK) and the downstream factor cFos (Kang *et al.* 2005), indicating a strong likelihood of activating mitogen-activated protein kinase kinase (MEK)/ERK signalling, as well as being able to modulate WNT signalling (Ding *et al.* 2010). These pathways are crucial for maintaining the stemness of the cells *in vitro*. Without such supplementation, these cells cannot be maintained *in vitro*.

Another pluripotent stem cell population can be derived from later-stage mouse blastocysts containing the EPI called EpiSCs (Brons *et al.* 2007), which have similar features to hESC in terms of their cell signalling (Greber *et al.* 2010). However, despite their ability to form multiple lineages *in vitro* and teratomas *in vivo*, the ability of EpiSCs to form chimeras is low and there

are no reports of germline transmission (Ng and Surani 2011). This research sparked the proposal that different pluripotent stem cell states can exist in culture, which may be attributed, in part, to the stage of embryo development they are derived from (Han *et al.* 2010). Recently, it was confirmed that hESC are derived from a post-ICM intermediate (O'Leary *et al.* 2012). However, intermittent states or reversion from one pluripotent state to another can also be obtained simply by manipulating the *in vitro* culture systems or by overexpression of factors (Zhou *et al.* 2010; Bernemann *et al.* 2011; Berge *et al.* 2011; Gu *et al.* 2012). This has been eloquently shown recently with the discovery that mESC in culture can spontaneously revert to a totipotent cell state reminiscent of a 2-cell blastomere (termed the '2c state'), lacking *Oct4*, *Nanog* and *Sox2* expression (Macfarlan *et al.* 2012). This suggests that culture conditions play a significant role in manipulating the growth and stem cell state of embryo-derived stem cells.

Porcine embryonic cell signalling differs to that observed in mouse and human embryos

Differences in early porcine embryo development and implantation exist compared with mouse and human embryonic development, and these differences have already been well addressed in recent reports (Hall 2008; Bazer *et al.* 2009; Oestrup *et al.* 2009). Given that morphological differences exist, it seems especially rational to consider that cell signalling differences also exist. In fact, reports do show that differences occur at both the transcriptional (Kuijk *et al.* 2008; Hall *et al.* 2009, 2010; Oestrup *et al.* 2009) and epigenetic levels in preimplantation porcine embryos compared with other species (Gao *et al.* 2010, 2011*b*). Given that the composition of most currently used media for the culture of pESCs and iPSCs is based on media used for the culture of either mouse or human ESC/iPSC, it also seems logical to assume that media composition may not be optimal for the porcine. Only a few studies have evaluated cell signalling events related to pluripotency in the preimplantation porcine blastocyst (Kuijk *et al.* 2008; Hall *et al.* 2009, 2010; du Puy *et al.* 2011; Wolf *et al.* 2011*c*). Therefore, it is evident that determining whether the FGF and/or LIF pathway, or other important regulatory pathways, are active in porcine pluripotent cells is critical to refine media composition effectively and/or to block the predisposition of these cells to form neural cells (Puy *et al.* 2010).

Studies that outline the key cell signalling events in the pluripotent populations of the porcine embryo will lead to improvements in cell culture media and, ultimately, improvements in the culture of pluripotent cells derived from the embryo. To date, only a few studies have addressed this. The LIF-activated JAK/STAT pathway has been shown not to be important in the pluripotent ICM and EPI in the pig due to the absence of a LIF receptor (Hall *et al.* 2009). However, the downstream activator STAT3 is expressed (Hall *et al.* 2009), which can also be activated by other pathways, such as the WNT pathway in mESC (Hao *et al.* 2006). Furthermore, *FGFR1* has been shown to be exclusively expressed in the EPI, whereas bFGF is produced and likely secreted from the TE, indicating that FGF signalling may occur in the pluripotent EPI (Hall *et al.* 2009). However, researchers who have attempted to culture pESC in the presence of either FGF, LIF or a combination of both have not had any success (Moore and Piedrahita 1997; Wianny *et al.* 1997; Brevini *et al.* 2007*b*), revealing that supplementation of these growth factors alone does not support pluripotency.

Major reprogramming factors (OCT4, NANOG and SOX2) are expressed differently in early porcine compared with mouse and primate embryos

The major transcription factors associated with pluripotency in the mouse and human, namely OCT4, NANOG and SOX2, have been well studied in the porcine ICM and EPI, revealing that OCT4 is expressed in both the ICM and TE of the prehatched blastocyst (Vejlsted *et al.* 2006; Hall *et al.* 2009). Interestingly, OCT4 expression in the TE has also been demonstrated in human preimplantation embryos, where POU5F1_1A, an isoform of OCT4 (expressed in hESC), is localised in the nucleus of both the TE and ICM and is also expressed in hESC (Cauffman *et al.* 2006). The expression of OCT4 has also been observed in both the ICM and TE of primate blastocysts (Harvey *et al.* 2009), showing the pig has greater similarity in expression to primate rather than mouse embryos.

Studies in porcine Embryonic day 5–6 embryos have also shown that the blastocyst containing the ICM lacks NANOG and SOX2 (Hall *et al.* 2009); however, slightly later embryos containing the early epiblast (Embryonic day 8.5) express both NANOG and SOX2 (du Puy *et al.* 2011). This differs considerably from both mouse and primate embryos. In the primate blastocyst, it has been shown that NANOG expression precedes OCT4 expression in the ICM (Harvey *et al.* 2009). It is only in the porcine EPI (in Embryonic day 10 embryos) that exclusive expression of OCT4, NANOG and SOX2 is observed (Hall *et al.* 2009; du Puy *et al.* 2011).

Together, these data suggest that the pig embryo has a unique expression profile in the blastocyst containing the ICM that differs to that in both primate and mouse embryos, but that the expression of these transcription factors is similar in the later EPI. Therefore, whether OCT4, NANOG and SOX2 are useful in assessing pluripotent ICM cultured cells is questionable. What remains unknown is whether the cells cultured *in vitro* can be maintained in the ICM or post-ICM state. Research to date suggests that cultured porcine ICM cells derived from early blastocysts do express *OCT4*, *NANOG* and *SOX2* in early passages, which may suggest that the cells could enter a post-ICM state *in vitro* (du Puy *et al.* 2011; Wolf *et al.* 2011*a*).

Expression of cell surface markers in the porcine EPI and TE compared with mouse and human embryos and ESCs

Cell surface markers have been used to characterise both mESC and hESC, and include stage-specific embryonic antigen (SSEA)-1 (in the case of mESC) and SSEA-3, SSEA-4, TRA-1–60 and TRA-1–81 (in the case of hESC). The expression of these cell surface markers has been shown to differ between the two species both in pluripotent cells (Table 1) and embryos (Table 2). We have observed that the porcine early blastocyst (Embryonic day 5–6) expresses SSEA-1 in both the ICM and TE

Table 1. Expression of cell surface markers previously described in porcine, murine and human embryonic stem cells (ESC), induced pluripotent stem cells (iPSC) and epiblast stem cells (epiSC)

ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; epiSC, epiblast stem cell; +, expression; −, lack of expression; N/D, undetermined; SSEA, stage-specific embryonic antigen. Asterisks indicate weak expression

Marker	ESC			iPSC			epiSC	
	Porcine	Murine	Human	Porcine	Murine	Human	Porcine	Murine
SSEA-1	+[D]	+	−	−[B]/+[C]	+[A]	−[A]	+[F]	+[E]
SSEA-3	N/D	−	+	+[B]/−[C]	−[A]	+[A]	N/D	N/D
SSEA-4	+[*D]	−	+	+[B]/+[*C]	−[A]	+[A]	+[F]	N/D
TRA-1–61	N/D	−	+	+[B]/−[C]	−[A]	+[A]	N/D	N/D
TRA-1–81	N/D	−	+	+[B]/−[C]	−[A]	+[A]	N/D	N/D

Data are from: [A]Maherali and Hochedlinger (2008); [B]Wu *et al.* (2009); [C]Ezashi *et al.* (2009); [D]ESC derived from upregulation of KLF4 and OCT4 (Telugu *et al.* 2011); [E]Brons *et al.* (2007); [F]Alberio *et al.* (2010).

Table 2. Expression of cell surface markers in porcine, murine and human blastocysts

+, expression; −, lack of expression; N/D, undetermined; SSEA, stage-specific embryonic antigen

Marker	Inner cell mass			Epiblast			Trophectoderm					
							Early blastocyst			Hatching/hatched blastocyst		
	Porcine	Murine	Human	Porcine	Murine	Human	Porcine	Murine	Human	Porcine	Murine	Human
SSEA1	+[A]	+[B]	−[B]	−	+[B]	−[B]	−[A]	+[B]	+[B]	+	+[B]	+[B]
SSEA3	N/D	−[B]	+[B]	−	−[B]	+[B]	N/D	−[B]	−[B]	+	−[B]	−[B]
SSEA4	N/D	−[B]	+[B]	−	−[B]	+[B]	N/D	−[B]	−[B]	+	−[B]	−[B]
TRA–1–61	N/D	−[B]	+[B]	−	−[B]	+[B]	N/D	−[B]	−[B]	+	−[B]	−[B]
TRA–1–81	N/D	−[B]	+[B]	−	−[B]	+[B]	N/D	−[B]	−[B]	+	−[B]	−[B]

Data are from: [A]Hall *et al.* (2010); [B]Henderson *et al.* (2002).

(Hall *et al.* 2010); however, this expression is lost in the developing EPI (Hall *et al.* 2010). Instead, SSEA-1 is detected only in the TE in the later embryo (Hall *et al.* 2010). This temporal change in expression as the porcine embryo develops recapitulates the two different stem cell states cultured *in vitro* of mESC (ICM state, which express SSEA-1) and hESC (post-ICM state, which lacks SSEA-1). We have also studied the expression of SSEA-3, SSEA-4, TRA-1–60 and TRA-1–81 in porcine embryos collected at Embryonic day 8–10 of development. Expression of these cell surface markers has only been studied in expanded and hatching mouse and human blastocysts containing the presumed ICM and/or EPI by one research group (Henderson *et al.* 2002; Table 2). In our study, the EPI appears different to that of both human and mouse embryos, expressing none of the cell surface markers within the pluripotent EPI; however, these markers are prominent in the surrounding TE (V. J. Hall and P. Hyttel, unpubl. obs.; Table 2). Further investigations are needed into the expression of SSEA-3, SSEA-4, TRA-1–60 and TRA-1–81 in the earlier porcine embryo. However, on the basis of these unpublished results, we see clear differences in this species, which suggests that the hatched pig embryo containing the EPI has an independent expression profile compared with hatching mouse and human embryos. Interestingly, expression of either SSEA-1 (Ezashi *et al.* 2009), SSEA-3, SSEA-4, TRA-1–60 and TRA-1–81 (Wu *et al.* 2009) or SSEA-4 (Esteban *et al.* 2009) has been observed in piPSC, illustrating that clear differences exist between the different cell lines produced. This may indicate dynamic differences in the cultured porcine stem cell state, which could be related to the reprogramming factors or culture conditions used.

Potential cell signalling pathways in the porcine ICM and corresponding TE

The porcine pluripotent ICM appears to express very few of the genes studied (Fig. 1). Despite cell pluripotency, the ICM does not appear to express SMAD genes, the WNT receptor *SFRP1* or *GSK3b*, but does express *c-Myc* (V. J. Hall and P. Hyttel, unpubl. data). A previous study reported that GATA6 was expressed in the porcine ICM only (Kuijk *et al.* 2008; this is in contrast with observations in the mouse embryo, in which GATA6 expression was also detectable in the TE (Koutsourakis *et al.* 1999). With the absence of NANOG and SOX2 at this stage of development, the key regulators of pluripotency are largely unknown, but may possibly include c-Myc. Further investigation of JAK/STAT signalling is required in the porcine ICM to better understand the events that govern cell renewal. Very little is also known about signalling in the TE at this stage. However, CDX2 expression has been determined in the TE of *in vitro*-produced blastocysts (Kuijk *et al.* 2008; Fig. 1). Interestingly, ultrastructural imaging of the early porcine blastocyst containing the ICM indicates little transcription may be occurring based on the presence of

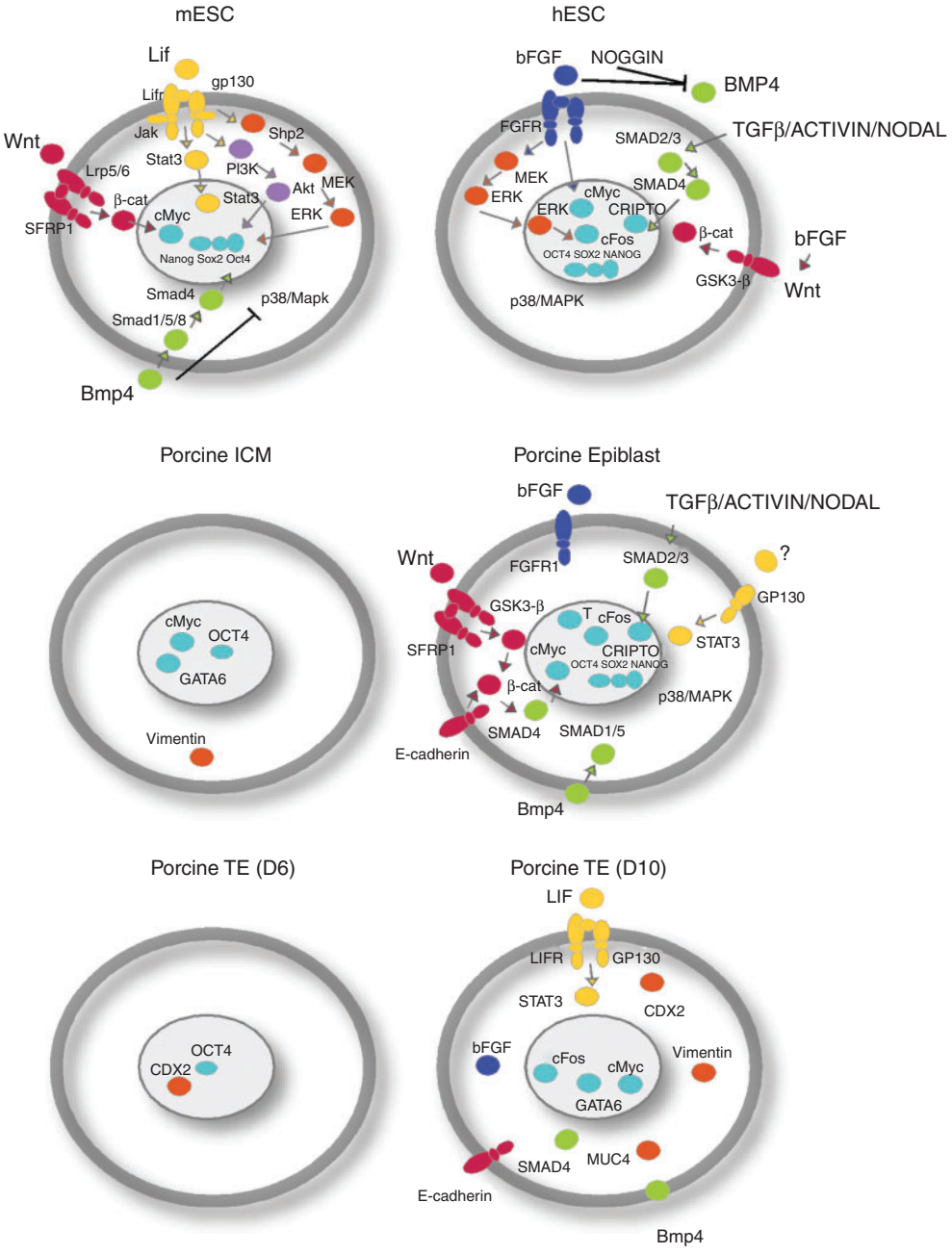

Fig. 1. Postulated cell signalling events in the porcine inner cell mass (ICM), epiblast (EPI) and trophectoderm (TE) compared with mouse embryonic stem cells (mESC) and human embryonic stem cells (hESC) indicates that the porcine blastocyst expresses few genes related to WNT and bone morphogenetic protein (BMP) signalling, whereas the pluripotent EPI likely has an active fibroblast growth factor (FGF), WNT, BMP and transforming growth factor (TGF)-β/ACTIVIN/NODAL signalling pathway. Little is known about the expression of the porcine TE from the Day 6 blastocyst; however, basic (b) FGF is produced from the TE of the Embryonic day 10 embryo and the leukaemia inhibitory factor (LIF) signalling pathway may be active, given the observed expression of *LIF receptor* (*LIFR*), *Glyocprotein 130* (*GP130*) and *Signal transducer and activator of transcription 3* (*STAT3*).

small mitochondria with only few cristae, small Golgi complexes, sparse smooth and rough endoplasmic reticulum and rare observations of mitosis (Hall *et al.* 2010), which may indicate that, at this stage of embryo development, the pluripotent cells may enter a short period of dormancy or a rest state. This could explain, in part, why few genes are expressed. Transcriptional profiling of these cells could help reveal other genes important during this stage of development.

Potential cell signalling pathways in the porcine EPI and corresponding TE

Investigations into various genes associated with pluripotency reveal that the EPI expresses many of these genes compared with expression in the earlier ICM. We have found that the *SMAD* genes and genes associated with BMP cell signalling are expressed in the porcine EPI (V. J. Hall and P. Hyttel, unpubl. data). Expression of *SMAD1*, *SMAD2*, *SMAD3* and *SMAD5* can be detected in the isolated EPI from the later Day 10 embryo, which may indicate that these genes are transcribed. Furthermore, *SMAD4* and *BMP4* can be detected in pooled EPIs of the same developmental stage. In the case of WNT signalling, the *SFRP1*, *GSK3b* and β-catenin genes can be detected in the porcine EPI, but that only the gene encoding β-catenin can be detected in the earlier porcine blastocyst. These observations indicate the presence of active WNT signalling only in the later-stage pluripotent EPI. In the case of JAK/STAT signalling, *GP130* and *STAT3* are expressed in the porcine EPI, indicating that JAK/STAT signalling is present but is not activated by LIF (V. J. Hall and P. Hyttel, unpubl. data). These observations, together with previously published reports (Kuijk *et al.* 2008; Hall *et al.* 2009, 2010; Wolf *et al.* 2011*b*, 2011*c*), reveal several different potentially active pathways in the EPI (Fig. 1). At this point, little is known about TGF-β and BMP receptor expression and therefore receptors for these pathways are not shown in Fig. 1. In summary, the porcine EPI appears to exhibit a more 'active' state of pluripotency compared with the porcine ICM, in which more commonly associated cell signalling genes of pluripotency are expressed. It appears that FGF signalling is involved, given the expression of *FGFR1*, however information regarding the expression of other FGF receptors is required. Given that bFGF is expressed and likely secreted from the porcine TE (Hall *et al.* 2009), this could be a significant proximal source for governing cell proliferation.

Very little is known about the genes expressed in the TE of porcine preimplantation embryos. However, we have found that the TE expresses *BMP4* and *SMAD4*. In addition, the TE expresses *LIFR*, *GP130* and *STAT3*, indicative of a likely active JAK/STAT signalling pathway activated by LIF (V. J. Hall and P. Hyttel, unpubl. data). Although the TE did not express *FGFR1*, it was found to express *c-Myc* (V. J. Hall and P. Hyttel, unpubl. data). Whyte and Stewart (1989) have detected *c-Fos* expression in the TE at this stage, and *CDX2* and *GATA6* are expressed in the TE of Embryonic day 10 embryos (Gao *et al.* 2011*a*), which suggests that these genes associated with differentiation of the TE in the mouse, may likely be conserved (Koutsourakis *et al.* 1999; Strumpf *et al.* 2005). An overview of known genes and potentially active pathways in the TE of both Day 6 and Day 10 porcine embryo is shown in Fig. 1 based on our own unpublished findings and previously published reports (Hall *et al.* 2009).

Conclusion

In summary, this paper provides an overview of known gene expression in the developing porcine preimplantation embryo, specifically that of genes expressed in the pluripotent ICM and EPI and corresponding TE. Few genes associated with pluripotency in mouse and human have been found in the early porcine embryo containing the ICM and, despite uncovering potentially active pathways in the porcine EPI, little progress has been made in establishing a stable pESC, because neither bFGF nor LIF can support the long-term growth of pESC *in vitro*. Transcription profiling could help uncover novel transcription factors and cell signalling pathways that regulate pluripotency in the ICM and help determine more about the cell signalling pathways in the EPI. Furthermore, the addition of cell signalling inhibitors to cultured piPSC and embryonic cells could help reveal which cell signalling pathways are crucial in the regulation of cell renewal and proliferation. The composition of the culture media has been shown in many recent studies to impact significantly on the *in vitro* stem cell state. Pluripotent iPSC can form neural stem cells during a transient period after reprogramming when cultured in a neural medium (Kim *et al.* 2011) and hESC can form mESC-like cells simply by altering the culture conditions (Gu *et al.* 2012). Thus, determining the cell signalling cascades and the activators of these cascades in the porcine ICM and EPI will help improve culture conditions, which could lead to future establishment of bona fide pluripotent cells and stable iPSC.

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